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DEVELOPMENT OF A PARADIGM TO ASSESS NUTRITIVE AND BIOCHEMICAL SUBSTANCES IN HUMANS: A PRELIMINARY REPORT ON THE EFFECTS OF TYROSINE UPON ALTITUDE- AND COLD-INDUCED STRESS RESPONSES

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SUMMARY

Tyrosine, a large neutral amino acid found in food, is the precursor for the catecholamine neurotransmitters dopamine and norepinephrine. Recent experiments have shown the behavior of animals given tyrosine is less impaired after stressful treatments than that of animals given placebo. Various environmental stressors are known to deplete central catecholamine stores and tyrosine's positive behavioral effects in animals are associated with reversal of this neurochemical deficit. Therefore, we investigated whether tyrosine administration would reduce adverse behavioral and physiological effects in humans induced by two combined environmental stressors, hypoxia and cold.

Twenty-seven young male military volunteers were tested in a double-blind crossover design. They were tested once with placebo and once with tyrosine at a control condition (550 m + 22°C) and at two levels of multiple environmental stressors (4200 m + 15°C and 4700 m + 15°C). A divided dose of 100 mg/kg of tyrosine or placebo was given 15 min before ascent to altitude and 50 min later. Behavioral assessment (a battery of performance tests and symptom and mood questionnaires) was begun 85 min after the initial dose and testing continued at specified times for the next 3 3/4 hours. Performance tests evaluated simple and choice reaction time to visual stimuli, vigilance, and processing of symbolic, numerical, verbal, and spatial materials. Three mood scales, a symptom questionnaire, and a sleepiness scale were also used. Blood samples were taken just before the first dose of tyrosine was given and again 150 and 265 min later. The samples were analyzed for plasma tyrosine and cortisol concentrations.

Performance, symptoms, and mood were adversely affected by both levels of high altitude and cold. Tyrosine administration in this exploratory study appeared to minimize the adverse consequences of these stressors. Tyrosine enhanced performance (e.g. complex information processing, vigilance, and reaction time) and reduced subjective symptoms (coldness, muscle discomfort, and headache). Mood states (e.g. anxiety, tension, and clear thinking) were also improved. Tyrosine had more beneficial effects at progressively more stressful altitude and cold conditions. Further research is necessary to replicate and extend the findings from this exploratory study.

MILITARY RELEVANCE

Future military operations will present unprecedented challenges. Combat is likely to be intensive, quick-paced, and fought day and night. Dramatic sleep loss, altered work-rest cycles, uncertainty, and misinformation will also be commonplace. Troops are also likely to be rapidly deployed to environments very different from that to which they are acclimated.

Military planners, doctrine developers, and commanders formulate strategies to sustain performance under a variety of adverse conditions. Special equipment, clothing, training, team building, and doctrine are some measures initiated to meet the challenges of varied combat theaters. In this study tyrosine, an amino acid in animal protein foods, was evaluated for its possible beneficial effects in a stressful situation, i.e. altitude and cold challenge. Such studies may aid the soldier by demonstrating nutritional substances can enhance his capabilities in stressful situations.

INTRODUCTION

Studies in rodents have demonstrated that some adverse physiological and behavioral effects of acute stress can be ameliorated by tyrosine, administered either in an acute dose or in the diet (1-3). Tyrosine is a constituent amino acid of many protein foods and is the precursor of the catecholamine neurotransmitters dopamine and norepinephrine (4). When tyrosine is administered in sufficient quantities, it can increase brain catecholamine concentration and turnover (4). When catecholaminergic

neurons are highly active they release more neurotransmitters (dopamine or norepinephrine), and therefore more substrate (tyrosine) is required. Since norepinephrine-containing neurons of the locus coeruleus have been shown to modulate alertness, activity and anxiety levels, the behavioral deficits associated with acute stress have been attributed to the depletion of norepinephrine in these neurons (5,6).

Animals that are acutely stressed display a combination of behavioral, cardiovascular and neurochemical changes (7). Behaviorally, they respond less to their environment, explore less, and generally seem debilitated. Simultaneously, brain norepinephrine turnover increases substantially and norepinephrine stores may be depleted. Tyrosine, given either systemically just before the stressful situation or as a dietary supplement, protected treated animals from both the neurochemical and the behavioral changes associated with tail shock and cold swim stressors (1-3). Tyrosine also lowers blood pressure in spontaneously hypertensive rats, subjected to stressful experimental conditions (8).

To date, we are aware of only a few studies where tyrosine has been administered to normal human subjects, although it may reduce depression in certain subgroups of depressed patients (9). In studies with normal subjects (10-12) no adverse effects of tyrosine administered in doses as large as 150 mg/kg were noted. In fact, two of these studies (11,12) showed a small improvement in mood and responsiveness following acute tyrosine administration. However, subjects in these studies did not experience experimental stressors, and it is under stressful conditions that tyrosine would be expected to have its greatest effects on behavior.

This study evaluated the benefits of administering tyrosine to humans during exposure to altitude and cold stressors. Altitude with cold has been shown to produce measurable impairments in affect and performance, important prerequisites for demonstrating a tyrosine effect. Also, hypoxemic effects should be maximal soon after ascent to altitude since previous studies (e.g. 13) suggested that cognitive performance impairments occurred at a simulated altitude of 4500 m (15,000 ft) within 60 min.

METHOD

SUBJECTS

Twenty-seven fully-informed medical research volunteers from Fort Detrick, MD, and the Natick Research Development and Engineering Center (Natick, MA) were subjects. The duration of their military service varied from a few weeks to over 9 years. This was the first research study for some subjects; others had served in many. About two-thirds of the subjects had not experienced high altitude before and none had participated in a tyrosine experiment. All subjects were given physicals; no subjects had medical histories that would contraindicate altitude and cold exposure.

ASSESSMENT METRICS

Behavioral (See Table I) and biochemical indices were used to evaluate cognitive performance, arousal, mood, symptoms, and responses to stress. These measures were collected using various media: 1) paper and pencil, 2) computer cards for Q-sort task, 3) portable computers with electroluminescent displays (Grid Compass II, Model 1131), and 4) blood samples.

Cognitive Tasks. Cognitive performance was assessed with the Addition, Coding, Map Compass, Number Comparison, Pattern Recognition and Tower Tasks. Sample items for each task are shown in Fig. 1. The Map Compass and Tower Tasks were developed in our laboratory (14); whereas, the remaining tasks were developed in the Navy's Performance Evaluation Tests for Environmental Research (PETER) program (15,16). All tasks were generated on a computer and printed, off-line, on a laser copier. Each performance task had 15 alternate forms. All tasks were previously shown to be sensitive to high altitude and/or other stressors (13,17).

TYPE OF TEST	TEST/SCALE/QUESTIONNAIRE	MEDIUM
COGNITIVE	ADDITION	PAPER & PENCIL
	CODING	PAPER & PENCIL
	MAP COMPASS	PAPER & PENCIL
	NUMBER COMPARISON	PAPER & PENCIL
	PATTERN RECOGNITION	PAPER & PENCIL
	TOWER TASK	PAPER & PENCIL
REACTION TIME	SIMPLE VISUAL REACTION TIME	PORTABLE COMPUTER
	FOUR-CHOICE VISUAL REACTION TIME	PORTABLE COMPUTER
VIGILANCE	DUAL TASK INFORMATION PROCESSING	PORTABLE COMPUTER
SYMPTOMS	ENVIRONMENTAL SYMPTOMS QUESTIONNAIRE	PORTABLE COMPUTER
MOOD STATE	CLYDE MOOD SCALE	Q-SORT (COMPUTER CARDS)
	MULTIPLE AFFECT ADJECTIVE CHECK LIST	PAPER & PENCIL
	PROFILE OF MOOD STATES	PAPER & PENCIL
	STANFORD SLEEPINESS SCALE	PAPER & PENCIL

Table I. Performarce, symptom, and mood scales used ir the study.

These performance tasks require cognitive processes inherent in many real-world tasks. For example, Map Compass requires association of direction and degree relationships, conceptualization of changing spatial relationships, and the ability to calculate distance or new grid coordinates. The Coding task requires that subjects write unique symbols for different numbers from the legend at the top of the page. This task is similar to manual procedures for encoding sensitive military communications. Subjects performing the Number Comparison Task indicate if two numbers are the same or different, much like comparing part numbers, grid coordinates, or numbers on property inventories. Pattern Recognition requires choosing a pattern (histogram), from an array of eight patterns, that is identical to the sample pattern. This is like recognizing computer programming, electronic, or map symbols. Subjects evaluate spatial problems on Tower Task with a series of learned algorithms and choose an appropriate strategy (14). Subjects are evaluated on their decisions, i.e. Is the problem "possible"? Is the problem "optimal"?.

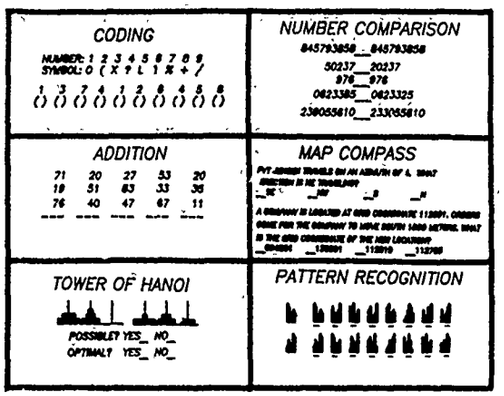

Figure 1. Sample items from the cognitive performance tasks.

The mental processes are like those for assembling mechanical components on a transmission or carburetor.

Reaction Time & Vigilance Tests. The Simple Visual Reaction Time (Simple RT), Four-Choice Visual Reaction Time (Choice RT), and Dual Task Information Processing Tasks (Dual-Task Vigilance) were administered on the Grid computers. After the presentation of a visual cue on the screen, the subject responds as quickly as possible in the Simple RT task. Such trials are repeated several times. Choice RT resembles the Wilkinson four-choice reaction time task and measures visual vigilance (18). On each trial a subject is presented a visual stimulus at one of four locations on the computer display. The subject strikes one of four adjacent keys on the keyboard to indicate the location of the stimulus.

Subjects simultaneously perform two tasks in the Dual-Task Vigilance test. One of the tasks is a modified version of the Bakan vigilance test (19); the other requires the "estimation of two classes of events in a signal stream". Our version of the Bakan test presents a series of three-digit numbers on the computer screen every 2.25 sec for 30 min. Each number usually differs from the previous number by one digit; however, occasionally all three digits are repeated. A subject's task is to detect such an occurrence. For the signal estimation test a single letter or digit is presented simultaneously on the display, to the right of the Bakan stimulus. Occasionally, the presentation of all stimuli is halted and subjects estimate the proportion of letters in the most recent series of stimuli. The proportion actually varies randomly from 0.2 to 0.8.

Symptom Questionnaire. Symptoms and subjective states were measured with the Environmental Symptoms Questionnaire (ESQ) as developed by Sampson (20). The ESQ was administered on the Grid Computer. It is a 67 statement questionnaire where each item is rated on a 6-point scale. Typical statements include: "I feel lightheaded", "I feel weak", and "I feel good". The ESQ factor structure is shown in Table II.

Clyde Mood Scale (CMS)
Friendliness
Aggressiveness
Clear Thinking
Sleepiness
Unhappiness
Dizziness
Environmental Symptoms Questionnaire (ESQ)
Cerebral (Acute Mountain Sickness)
Respiratory (Acute Mountain Sickness)
Ear, Nose, and Throat
Coldness
Distress
Alertness
Exertion
Muscular Discomfort
Fatigue
Multiple Affect Adjective Check List (MAACL)
Anxiety
Depression
Hostility
Profile of Mood States (POMS)
Anger
Confusion
Depression
Fatigue
Tension
Vigor
Stanford Sleepiness Scale (SSS)
Sleepiness

Table II. Subscales (factors) for the mood, symptom, and sleepiness questionnaires.

Mood Scales. The Clyde Mood Scale (CMS), Multiple Affect Adjective Check List (MAACL), the Profile of Mood States (POMS), and the Stanford Sleepiness Scale (SSS) were used to assess subjects' moods and arousal states. The factor structures for each of these scales are shown in Table II. The CMS consists of 48 adjectives, e.g. "good-natured", "troubled", "lonely", "impulsive", which are rated on a discrete anchor point scale ("not at all", "a little", "quite a bit", and "extremely"). The CMS was administered on computer cards as a Q-sort task for automated scoring (21). The MAACL has 132 adjectives such as "devoted", "healthy", "mild", and "panicky"; it is a paper and pencil mood scale (22). Subjects check those adjectives that apply to them. The POMS is also a paper and pencil scale; it has 65 adjectives (e.g. "bitter", "trusting", "lively") that are rated on a 5-point scale ranging from "Not At All" to "Extremely" (23). The POMS has been employed in many psychopharmacological studies and is sensitive to the effects of many different classes of psychoactive drugs, including hypnotics and stimulants. The Stanford Sleepiness Scale (SSS) consists of seven statements on a sleepiness-alertness continuum (24).

Biochemical Indices. Tyrosine was assayed after the methods of Shen and Abell (25) in 50 ul of plasma and using 200 ul of phenylalanine ammonia lyase (EC 4.3.1.5) in a total volume of 1.5 ml (0.1M Tris buffer, pH = 8.75) at 315 nm. Plasma cortisol levels were determined using radioimmunoassay test kits and techniques described in the technical bulletin (New England Nuclear Corporation, N. Billerica, MA). Normal values for adult male test subjects range from 4-24 ug.dl^{-1} using these techniques.

PROCEDURES

Experimental Design. Each group of subjects was tested over a 20 day interval. All training and testing sessions were in the altitude chamber at the U.S. Army Research Institute of Environmental Medicine, Natick, MA. The first week (Monday-Friday) subjects were trained and practiced on the various tests, scales, and questionnaires. On Friday subjects were also given a 50-min altitude orientation, e.g. shown how to open "ear (eustachian tube) blocks" during simulated altitude ascent or descent. This session also included an ascent to 4200 m for 15-20 min. Monday, Wednesday, and Friday of the second and third weeks were devoted to experimental testing. On a test day approximately half

of the subjects received tyrosine; the other half, placebo. The ordering of environmental conditions within a test week were roughly counterbalanced over the entire study. The environmental conditions for a group of subjects were identical on a given day, e.g. Wednesday, of both experimental weeks.

Subjects were tested in three groups (10, 5, and 12 subjects) in Jan., Feb., and late July 1986. Each group was always tested together, seated on metal chairs around two square tables. The schedule of activities, test sequence, time after initial ingestion of tyrosine, and time after ascent to altitude is shown in Table III. This schedule was the same for all groups. Subjects wore the Army's battle dress uniform to control clothing (heat loss) variables. The day before each test day subjects were told to refrain from alcohol; that evening they were housed in a special dormitory beginning at 2200 hours.

TIME (H)	ACTIVITY	MINUTES AFTER 1ST TREATMENT	MINUTES AFTER ASCENT	ASSESSMENT INSTRUMENTS
0535-0540	WAKE-UP CALL			
0540-0630	SHOWER & SHAVE			
0630-0650	SPECIAL BREAKFAST			
0650-0700	GO TO ALTITUDE CHAMBER			
0700-0715	BLOOD PRESSURE 1			
0715-0730	BLOOD 1, TREATMENT 1	0		
0730-0740	ASCENT TO ALTITUDE	15	0	
0740-0805	RELAXATION TIME 1	30	15	
0805-0820	TREATMENT 2	50	35	
0820-0840	BLOOD PRESSURE 2	70	55	
0840-0910	COGNITIVE TESTS	95	80	PRI.ADI.CDI.NCI.MCI, TVI
0910-0930	REACTION TIME & MOOD TESTS	120	105	CHOICE RT, POMS1, SSS1
0930-0940	COGNITIVE TESTS	135	120	MN2, AR2
0940-1005	BLOOD 2 & BREAK 1	150	135	
1005-1045	VIGILANCE & MOOD TESTS	185	170	POMS2, SSS2, DUAL TASK
1045-1100	COGNITIVE TESTS	210	195	CD2, NC2, MC2
1100-1115	BLOOD PRESSURE 3 & BREAK 2	225	210	
1115-1120	MOOD TESTS	235	220	CMS1 & MWCL1
1120-1140	REACTION TIME & MOOD TESTS	250	235	POMS3, SSS3, & SIMPLE RT
1140-1150	BLOOD 3 & BREAK 3	265	250	
1150-1205	SYMPTOM & COGNITIVE TESTS	275	260	CSQ1 & TVI
1205-1215	DESCENT FROM ALTITUDE	290	275	

Table III. Schedule of activities for an experimental test session.

Treatment. Tyrosine and placebo (cellulose) administration were double-blind. A total of 100 mg/kg of tyrosine was administered; this corresponds to about 80% of the daily dietary intake of tyrosine. Tyrosine or placebo was loosely packaged and administered in gelatin capsules (300 mg per capsule). The ordering of tyrosine or placebo for a subject was random for the first three treatment administrations with some restrictions. Ordering for the 4th, 5th, and 6th administrations was the opposite. Each subject received one-half of the total dose of tyrosine or placebo immediately before each test session. Approximately 50 min later each subject received the second divided dose. To minimize "first day" effects, all subjects ingested capsules (placebo) the fifth training session. Subjects were informed this was a test trial. For dietary control, subjects ate a light breakfast (apple or cranberry juice and two cereal bars) each morning soon after awakening. Decaffeinated coffee and water were also available.

Environmental Stressors. Environmental conditions on a test day were one of three conditions: 550 m + 22°C (1800 ft + 72°F), 4200 m + 15°C (13,800 ft + 59°F), or 4700 m + 15°C (15,500 ft + 59°F). The relative humidity was 30-50% and ventilation 0.71 cu m/min (25 cu ft/min). Environmental exposures were 280-290 min per day. Test subjects were not informed regarding the specific environmental conditions to be tested on a given day. During training ambient conditions were normobaric and thermoneutral (75 m + 22°C).

Cognitive Performance. All cognitive performance tasks were timed. Addition, Coding, Number Comparison, and Pattern Recognition were given for 3 min; Map Compass, for 4 min and Tower Task, for 6 min. Repeated testing procedures and methods were similar to those for the Performance Evaluation Tests for Environmental Research Program (13-16). Initially, subjects were given training and extensive practice with performance feedback. Feedback was no longer given after the 5th training day. All performance tasks were practiced repeatedly to insure performance was stable and near maximum. Each task had been completed 15 times before the subjects were evaluated in an experimental condition.

Reaction Time and Vigilance. After five warmup trials were given on Simple RT, 300 test trials were presented. Both errors of commission (responding before the visual stimulus is presented) and errors of omission (response latency >1 sec) were recorded. Five hundred Choice RT trials were administered in 10 min. Response latency and errors of omission and commission were recorded.

Subjects pressed a key on the Dual Task Vigilance Task when they detected a three-digit number on the display the same as the number just preceeding it. In addition, at six intervals (each 200 trials) the presentation of all stimuli was halted and subjects estimated the proportion of letters in the last series of 200 stimuli.

Mood and Symptoms. All mood and symptom scales were untimed. Subjects were instructed to "describe how you feel NOW" on each mood and symptom scale.

Biochemical Indices. A blood sample (< 20 ml) was taken from an antecubital arm vein of each subject using heparinized vacutainers just before ingestion of the first dose of tyrosine or placebo. A second and a third blood sample was withdrawn approximately 150 and 265 min later. Blood samples remained on ice during a sampling interval until aliquots from all test subjects were obtained. Samples were then centrifuged (4°C, 1500 g), the plasma separated, and aliquots prepared and frozen (-20°C) for subsequent assay. Tyrosine and cortisol were assayed.

Statistical Analyses. Separate analyses of each environmental condition compared the tyrosine and placebo outcomes (t-test, repeated measures). This strategy was chosen over an analysis of variance (across altitude conditions) because it allowed use of the maximum number of subjects in each analysis. Missing values occurred in the data base because approximately one-third of the subjects did not complete at least one test session due to ear infections, blocked sinuses, or other illnesses. Results are presented for all measures that detected statistically significant differences between tyrosine and placebo ($p \leq .05$) as well as tests that showed differences ($p \leq .15$). All comparisons are one-tailed.

A measure of cognitive performance was derived to reflect the combined effect of rate and accuracy changes, i.e. number of problems correct/min = (number of problems attempted - number of problems wrong)/min. In calculating this index a 2X weighting factor was applied to errors for Number Comparison and Tower Task to penalize for possible guessing on dichotomous responses. Corresponding weighting factors were applied to the Map Compass and Pattern Recognition tasks which have more than two response

alternatives.

Average reaction times for each subject were analyzed on the Simple RT and Choice RT tasks. Number of errors of commission was also analyzed on the Choice RT task. The number of critical stimuli correctly detected by each subject was analyzed on the Dual-Task Vigilance Task.

Unnormalized factor or raw scores were analyzed on the mood and symptom questionnaires.

RESULTS

Shown in Fig. 2 are plasma levels of tyrosine for each environmental condition for the initial subjects from groups 1 and 2. Values for subjects receiving tyrosine and subjects receiving placebo are shown before capsule ingestion and 150 and 265 min after

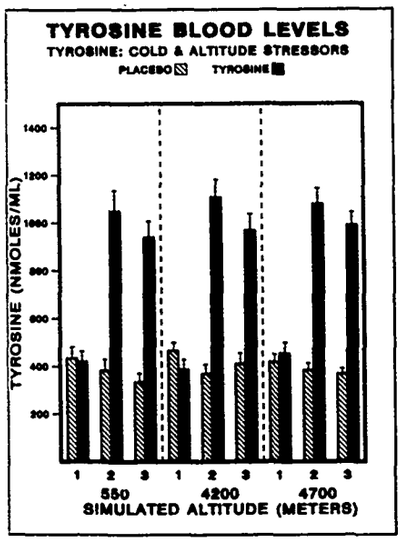

Figure 2. Circulating plasma tyrosine levels after tyrosine or placebo ingestion . Values for tyrosine- and placebo-treated subjects are shown for three levels of multiple environmental stressors (550 m + 22 °C, 4200 m + 15 °C, and 4700 m + 15 °C) and for three sampling intervals (before capsule ingestion, and 150 and 265 min later). The standard error of the mean is also indicated.

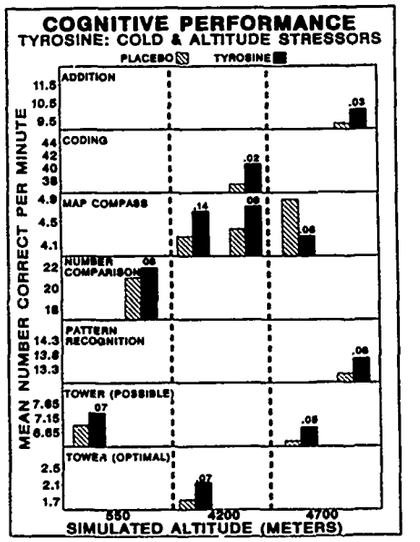

Figure 3. Performance on several cognitive tasks after tyrosine or placebo ingestion for three levels of multiple environmental stressors (550 m + 22 °C, 4200 m + 15 °C, and 4700 m + 15 °C). Actual probabilities for all treatment effects ($p \leq .15$) are shown. Tyrosine enhanced cognitive performance, except for Map Compass at 4700 m + 15 °C.

ingestion. These data indicate that after tyrosine ingestion a significant ($p \leq .001$) elevation in circulating tyrosine occurred in both subsequent blood samples; however, these increments were unaffected by either combination of altitude and cold. Tyrosine levels were highest in the second blood sample, taken approximately 150 min after tyrosine ingestion, and then fell slightly 265 min after tyrosine ingestion.

Preliminary data for circulating cortisol levels at sea level indicated there was a significant ($p \leq .05$) decrement in cortisol concentrations from the first to the third blood sample, irrespective of tyrosine ingestion. This decrement is consistent with the anticipated circadian reduction which ordinarily occurs between approximately 0700-1100h (26). Interestingly, for both altitude conditions, the trend toward decreasing cortisol levels is apparent, but the lowest levels attained, especially at the third sampling time, are not as depressed as in the sea level session. However, examination of the data for the 4700 m altitude (and cold) condition, at which physiological stress might be expected to be maximal, indicates no effects of tyrosine on cortisol levels; in fact, in both the second and third blood samples, cortisol levels in tyrosine-treated subjects are slightly elevated.

Fig. 3 shows the results from the cognitive performance tasks. Plotted values represent average performance levels at 550 m + 22 °C, 4200 m + 15 °C, and 4700 m + 15 °C that were different ($p \leq .15$) for the tyrosine- and placebo-treated subjects ; the exact probability for each comparison is shown above the solid bar (tyrosine). There were two

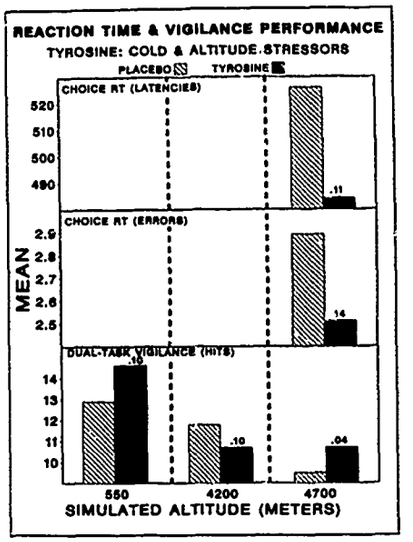

Figure 4. Reaction time and vigilance performance after tyrosine or placebo ingestion for three levels of multiple environmental stressors (550 m + 22 °C, 4200 m + 15 °C, and 4700 m + 15 °C). Actual probabilities for all treatment effects ($p \leq .15$) are shown. Performance was enhanced by tyrosine, except for Dual Task Vigilance at 4200 m + 15 °C.

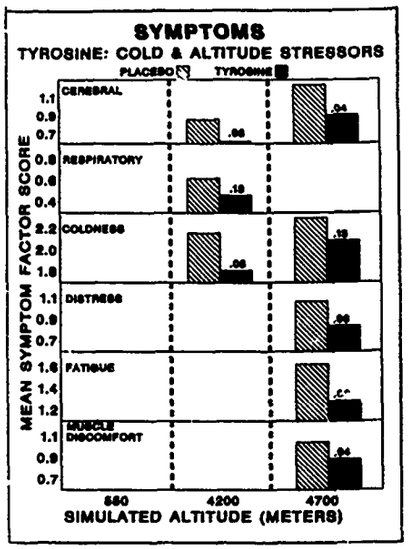

Figure 5. Self-rated symptoms after tyrosine or placebo ingestion. Actual probabilities for all treatment effects ($p \leq .15$) are shown. Tyrosine consistently reduced symptoms associated with the combination of environmental stressors (simulated high altitude and cold).

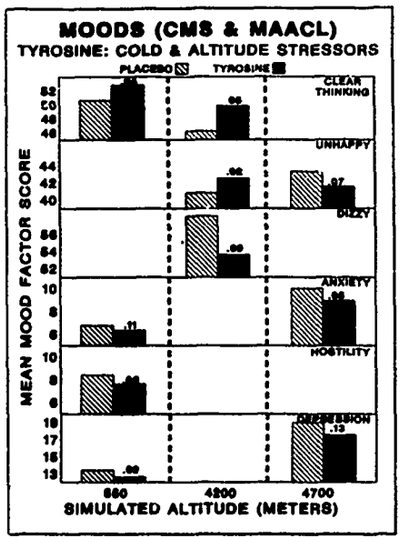

Figure 6. Self-rated mood states as measured by the Clyde Mood Scale (top three factors) and the Multiple Affect Adjective Check List (bottom three factors) after tyrosine or placebo ingestion. Actual probabilities for all treatment effects ($p \leq .15$) are shown. Tyrosine improved moods affected by the high altitude and cold conditions and the control environmental condition, except for the unhappiness factor at 4200 m + 15 °C.

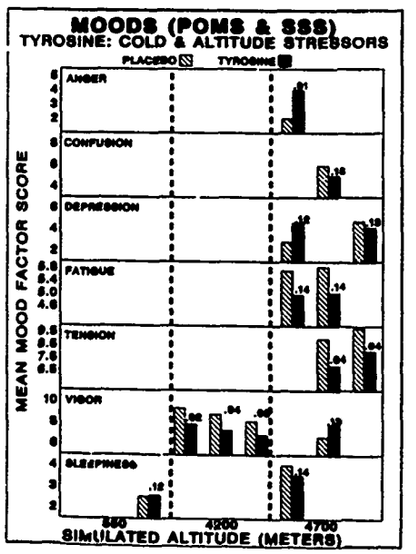

Figure 7. Self-rated mood states as measured by the Profile of Mood States (upper six factors) and Stanford Sleepiness Scale (lower factor) after the tyrosine or placebo ingestion. Actual probabilities for all treatment effects ($p \leq .15$) are shown.

MEASURE	550 m + 22°C p≤.05	p≤.15	4200 m + 15°C p≤.05	p≤.15	4700 m + 15°C p≤.05	p≤.15
COGNITIVE PERFORMANCE	0	2	1	4	2	4 (1)
REACTION TIME/VIGILANCE	0	1	0	1 (1)	1	3
SYMPTOMS (ESQ)	0	0	1	3	2	5
MOOD STATE (CMS & MAACL)	1	4	2 (1)	3 (1)	1	3
MOOD STATE (POMS & SSS)	0	1 (1)	2 (2)	3 (3)	3 (1)	10 (2)
TOTALS	1	8 (1)	6 (3)	14 (5)	9 (1)	25 (3)

Table IV. Performance, symptom, and mood treatment comparisons for tyrosine- and placebo-treated subjects. The number of statistically significant effects ($p \leq .05$), number of differences ($p \leq .15$), and instances when placebo was superior to tyrosine are shown; all comparisons were one-tailed. This analysis suggests the treatment effects are subtle in this exploratory study and may be hidden by measurement variability in our subject population.

test administrations per day, e.g. Map Compass showed a difference with 4200 m + 15°C for both the first and second test administrations. Enhanced performance is reflected in more problems correct/min. With the exception of the Map Compass data for 4700 m + 15°C (1st admin.) all treatment effects were in the expected direction. Beneficial tyrosine effects were demonstrated on all cognitive performance tasks for at least one environmental condition. Statistically significant ($p \leq .05$) treatment effects were seen on the coding task at 4200 m + 15°C and on the Addition and Tower Task (possible) at 4700 m + 15°C.

Fig. 4 shows the reaction time and vigilance performance measures. No evidence of treatment effects were observed for Simple RT. Reaction time and error measures on the Choice RT Task were decreased by tyrosine for the 4700 m + 15°C condition. Correct detections (i.e. hits) on the Dual-Task Vigilance Task increased with tyrosine at 550 m + 22°C and 4700 m + 15°C. Unexpectedly, Dual-Task Vigilance performance was impaired by tyrosine at 4200 m + 15°C. Increased Dual Task hits in tyrosine-treated subjects at 4700 m + 15°C were statistically significant ($p \leq .05$).

Fig. 5 shows the effects of tyrosine upon symptoms as measured by the ESQ. Tyrosine reduced symptom severity in every treatment-difference ($p \leq .15$). Cerebral discomfort, e.g. headache, and coldness were reduced by tyrosine at both 4200 m + 15°C and 4700 m + 15°C. Respiratory discomfort was also less at 4200 m + 15°C in soldiers given tyrosine. Distress, fatigue, and muscle discomfort at 4700 m + 15°C were also less in tyrosine-treated subjects. Statistically significant effects ($p \leq .05$) were seen for the cerebral and muscle discomfort factors at 4700 m + 15°C and for the coldness factor at 4200 m + 15°C. No tyrosine effects were found at sea level with this symptom questionnaire nor were there any treatment effects on the alertness, exertion, or ear-nose-throat factors (See Table II).

Fig. 6 shows tyrosine treatment effects as measured by the CMS (upper three factors) and the MAACL (lower three factors). Clear thinking was improved by tyrosine at 550 m + 22°C and 4200 m + 15°C, and the dizziness factor was improved at 4200 m + 15°C. Unhappiness factor scores were improved at 4700 m + 15°C by tyrosine but made worse at 4200 m + 15°C. The friendliness, aggressiveness, and sleepiness factors on the CMS did not exhibit any treatment effects.

Treatment effects were found with the MAACL at both 550 m + 22°C and 4700 m + 15°C; no mood factors were improved or made worse at 4200 m + 15°C (see lower part Fig. 6). Anxiety, hostility, and depression were reduced at 550 m + 22°C; anxiety and depression were also reduced at 4700 m + 15°C. Statistically significant beneficial effects ($p \leq .05$) were seen on the clear thinking scale (CMS) at 550 m + 22°C and 4200 m + 15°C and the anxiety scale (MAACL) at 4700 m + 15°C. Changes in unhappiness at 4200 m + 15°C were in the wrong direction ($p \leq .05$).

The POMS and SSS data are shown in Fig. 7. The first six factors, anger thru vigor, are for the POMS; sleepiness is for the SSS. Data are shown for three administrations of each scale at each environmental condition. Consistent with the other mood questionnaires, confusion, depression, fatigue, tension, and sleepiness were decreased and vigor was increased by tyrosine for the 4700 m + 15°C stressor condition on one or more administrations. A statistically significant ($p \leq .05$) beneficial effect was seen at 4700 m + 15°C on the tension scale. For unknown reasons some mood factors on the POMS detected changes inconsistent with the direction of our performance, mood, and symptom data above. Unexpectedly, depression and anger increased in tyrosine-treated subjects during the first administration of the 4700 m + 15°C environmental condition. Also, vigor was always decreased during the 4200 m + 15°C condition and sleepiness was increased at 550 m + 22°C.

Table IV summarizes the behavioral data by showing the number of times the treatment effects, i.e. placebo and tyrosine comparisons were different ($p \leq .05$ and $p \leq .15$) for each of the environmental stressor conditions. Unexpected instances of treatment-produced impairments are shown in parentheses. Increasing levels of environmental stressors resulted in more treatment effects, e.g. 1, 6, and 9 ($p \leq .05$) or 8, 14, and 25 ($p \leq .15$). Secondly, conventional significance levels, e.g. $p \leq .05$, resulted in 16 significant differences with four in the opposite direction from that expected for a beneficial tyrosine effect. The likelihood of 12 effects out of 16 (in the expected direction) being due to chance is less than 5 in 100. Examining data from the less stringent statistical level in the same manner yielded 47 treatment effects with 38 in the expected direction. The chances of this outcome being due to chance are less than 3 in 100,000.

DISCUSSION

To the best of our knowledge this exploratory study represents the first systematic attempt to determine if tyrosine has beneficial effects on humans exposed to stressful situations. The overall results tentatively suggest that tyrosine may improve certain aspects of performance and mood states under conditions of acute stress produced by simulated high altitude (hypobaric) and cold. Tyrosine's beneficial effects on performance were detected by several cognitive tests and tests of choice reaction time and vigilance. Tyrosine also reduced symptoms associated with acute exposure to hypoxia and cold stress such as fatigue, distress, and cerebral discomfort. Appropriate changes in mood state after tyrosine administration were also noted. For example, clear thinking was increased and dizziness and tension were decreased. Results obtained under the control environmental conditions suggest that tyrosine administration may have positive effects when minimal stress is present. Although potentially adverse effects of tyrosine on some mood states were observed on one of the three mood scales, i.e. the POMS, tyrosine generally produced improvements in performance, symptoms, and other mood states. The majority of mood changes detected (17 of 24, $p \leq .15$) were in the expected direction. Such improvements in mood were consistent with the beneficial effects of tyrosine on performance and symptoms concurrently observed. The occasional adverse effects on mood state that were noted on the POMS are difficult to explain since unlike the positive treatment effects detected, their pattern was not consistent across altitude conditions or other mood questionnaires.

The effects of tyrosine were greatest under the most adverse environmental conditions employed in this study, the simulated 4700 m and 15°C condition. For this condition, 22 treatment comparisons showed beneficial tyrosine effects ($p \leq .15$), i.e. reduced symptoms and improved performance and mood. Nine statistically significant improvements ($p < .05$) in performance and mood were observed at this altitude. Only one statistically significant adverse finding (anger factor on POMS) was noted.

Overall, the tyrosine effects observed were selective for the various behavioral parameters assessed, i.e. no effects on some mood factors (e.g. friendliness and aggressiveness) and performance tests (Simple RT). Treatment effects also appeared modest in magnitude. This is consistent with other studies which indicate that neurotransmitter precursors will produce more selective effects on behavior than drugs with similar properties (12,26). Actually, even high doses of psychoactive drugs rarely produce effects on all parameters assessed. Rather, effects are typically seen on a very limited number of dependent variables measured. This is probably the result of considerable differences in sensitivity across measurement instruments, as well as differences in the nature of the underlying parameters measured. It is particularly difficult to detect acute, effects of anti-anxiety agents. Consequently, in acute psychopharmacological studies with such drugs, relatively high doses must be administered and a large population sampled to detect reductions in anxiety (28). Additionally, the classic anti-anxiety agents, the benzodiazepines and meprobamate, typically impair task performance and reduce subjective alertness, although they do reduce self-reported anxiety (29). Based on our preliminary findings, tyrosine would appear to have a number of advantages compared to these drugs in some circumstances.

The relatively modest behavioral effects detected in this study may be the consequence of the relatively mild level of stress produced by our environmental conditions, the predictability of each daily test session, and the statistical power of our assessment instruments. Support for this is indicated by the smaller number of positive effects seen for the 4200 m + 15°C condition and by the modest changes in plasma cortisol produced by even the greatest altitude-cold condition. Preliminary calculations of statistical power suggest several of our tests would have marginal ability to detect treatment differences with the magnitude of the effects observed and our smaller number of subjects for some conditions (30). Also, it seems likely that some portion of the performance and mood impairments, observed for the combinations of hypoxia and cold, were attributable to reduced oxygen delivery to the brain or some other direct effect of altitude on central nervous system functioning. In fact, although cold stress has been reported to deplete catecholamine stores, hypoxia appears to have a relatively modest effect on central catecholamine function (7). Tyrosine would not be expected to reduce adverse effects directly attributable to central oxygen insufficiency. This may be critical since tyrosine would be most effective for alleviating the effects of an acute generalized stress response.

In general, this exploratory study indicates that tyrosine may be an appropriate intervention to enhance performance and mood states and decrease symptomatology in acutely stressful environments. While the beneficial effects of tyrosine we detected were limited, the adverse effects of other anti-anxiety agents on performance, such as the benzodiazepines, rule out their use in any situation where optimal task performance is required. Additionally, tyrosine may have fewer unwanted side effects since it is a nutrient that is present in substantial quantities in the diet.

Further research is necessary to determine optimal tyrosine doses, tyrosine's effects in other stressful situations, and the replicatibility of the preliminary findings from this exploratory study.

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ADDENDUM

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.